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Review

# Tribute to P. L. Lutz: a message from the heart – why hypoxic bradycardia in fishes?

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**Summary**

**The sensing and processing of hypoxic signals, the responses to these signals and the modulation of these responses by other physical and physiological factors are an immense topic filled with numerous novel and exciting discoveries. Nestled among these discoveries, and in contrast to mammals, is the unusual cardiac response of many fish to environmental hypoxia – a reflex slowing of heart rate. The afferent and efferent arms of this reflex have been characterised, but the benefits of the hypoxic bradycardia remain enigmatic since equivocal results have emerged from experiments examining the benefit to oxygen transfer across the gills. The main thesis developed here is that hypoxic bradycardia could afford a number of direct benefits to the fish heart, largely because the oxygen supply to the spongy myocardium is precarious (i.e. it is determined primarily by the partial pressure of oxygen in venous blood, $P_{v\dot{O}_2}$) and, secondarily, because the fish heart has an unusual ability to produce large increases in cardiac stroke volume ($V_{SH}$) that allow cardiac output to be maintained during hypoxic bradycardia. Among the putative benefits of hypoxic bradycardia is an increase in the diastolic residence time of blood in the lumen of the heart, which offers an advantage of increased time for diffusion, and improved cardiac contractility through the negative force–frequency effect. The increase in $V_{SH}$ will stretch the cardiac chambers, potentially reducing the diffusion distance for oxygen. Hypoxic bradycardia could also reduce cardiac oxygen demand by reducing cardiac d$P$/d$t$ and cardiac power output, something that could be masked at cold temperature because of a reduced myocardial work load. While the presence of a coronary circulation in certain fishes decreases the reliance of the heart on $P_{v\dot{O}_2}$, hypoxic bradycardia could still benefit oxygen delivery *via* an extended diastolic period during which peak coronary blood flow occurs. The notable absence of hypoxic bradycardia among fishes that breathe air during aquatic hypoxia and thereby raise their $P_{v\dot{O}_2}$, opens the possibility that that the evolutionary loss of hypoxic bradycardia may have coincided with some forms of air breathing in fishes. Experiments are needed to test some of these possibilities. Ultimately, any potential benefit of hypoxic bradycardia must be placed in the proper context of myocardial oxygen supply and demand, and must consider the ability of the fish heart to support its routine cardiac power output through glycolysis.**

Key words: heart rate, stroke volume, myocardial hypoxia, oxygen diffusion, coronary circulation, air breathing.

## Introduction

Peter Lutz was my first university mentor and then a colleague and friend for nearly 35 years. Peter was a remarkable scientist, one who was fascinated by biology in its broadest sense and who marveled at biological extremes. He sought answers to the question ‘why?’. He also realized that he could not always answer questions by himself and, I think, this is why he had an infectious enthusiasm for science. His enthusiasm stimulated others to find the answers, a tactic that certainly worked with me. Therefore, as a tribute to Peter’s generosity with ideas and his spirited willingness to throw down the gauntlet if challenged, I offer a synthesis of ideas on the unusual cardiac response of many fish to hypoxia – reflex bradycardia. Of course, this topic fits well with Peter’s interests in hypoxia and had sparked his curiosity because of the dichotomy that exists among vertebrates in terms of their cardiac response to environmental hypoxia.

## The evolutionary framework for the dichotomous cardiac response to environmental hypoxia

When environmental oxygen availability decreases, mammals respond by increasing external convection (lung ventilation), as well as increasing internal convection (cardiac output=$\dot{Q}$), primarily through tachycardia, so that tissue oxygen delivery is maintained despite a decrease in arterial oxygen

saturation. For many water-breathing fish the initial cardiac response to aquatic hypoxia is reflex bradycardia (Randall and Shelton, 1963; Butler and Taylor, 1971; Randall, 1982; Taylor, 1992) and not tachycardia as in mammals. This hypoxic reflex is well documented in both teleosts and elasmobranchs. It is triggered by externally located branchial oxygen receptors (Taylor, 1985; Burleson et al., 1992; Reid et al., 2006) (but see Sundin et al., 1999) and mediated by vagal cardio-inhibitory fibres. Systemic (dorsal aortic) blood pressure ($P_{DA}$) often increases in concert with hypoxic bradycardia, but the possibility of a baroreceptor reflex has been eliminated (Wood and Shelton, 1980). Severe hypoxia may directly depress cardiomyocytes, but this is true for both fish and mammals and is not considered further here. Also not considered here are the secondary effectors and modulators that may play additional roles during hypoxia, as indicated by the fact that bilateral branchial cardiac vagotomy will greatly attenuate, but not completely abolish, hypoxic bradycardia in the dogfish *Scyliorhinus canicula* (Taylor et al., 1977; Short et al., 1979).

From what we know of the hypoxic bradycardia response in extant vertebrates, it seems likely that it first appeared and then disappeared among the fish lineage. For example, hagfishes lack autonomic cardiac innervation (Nilsson, 1983) and heart rate ($f_H$) remains unchanged at around 23 beats min$^{-1}$ in response to severe hypoxia, although $\dot{Q}$ and cardiac stroke volume ($V_{SH}$) increase modestly (Axelsson et al., 1989; Forster et al., 1991). Similarly, a 24-h anoxic exposure has little effect on $f_H$ (Hansen and Sidell, 1983). Other water-breathing fishes, however, can decrease $f_H$ substantially during aquatic hypoxia. The level of bradycardia varies with the degree of aquatic hypoxia, temperature, and among species (Taylor et al., 1977; Wood et al., 1979b). Fish do possess cardio-accelerator mechanisms and so, in theory, they could respond to hypoxia with tachycardia like mammals. Fish can release vagal inhibitory cardiac tone to increase $f_H$, although in some situations a small vagal tone may leave little scope to increase $f_H$. In addition, positive chronotropic responses are possible through adrenergic stimulation *via* either elevated plasma catecholamine levels, or increased sympathetic innervation in certain teleosts (Nilsson, 1983; Farrell and Jones, 1992).

Hypoxic bradycardia is notably absent in some air-breathing fishes, hypoxia-tolerant fish and Antarctic teleosts. These exceptions are examined below. Given that lungfishes do not show bradycardia in response to aquatic hypoxia (Fritsche et al., 1993; Sanchez et al., 2001; Perry et al., 2005) and that lungfishes are considered the evolutionary forerunners of the tetrapods, the possibility arises that the evolutionary loss of hypoxic bradycardia may have coincided with some forms of air breathing in fishes.

The line of reasoning taken here is that natural selection has favoured hypoxic bradycardia because of some accrued benefit(s) for the fish, and these benefits are either unnecessary or unavailable to mammals. Even with a limited cardio-acceleratory capacity, bradycardia in response to hypoxia should not be needed unless there are benefits to the bradycardia. Therefore, the primary question addressed here is: What are the potential benefits of hypoxic bradycardia? To date the only experimental studies of potential benefits of hypoxic bradycardia have been those examining the benefit to gas transfer at the gills (Short et al., 1979; Taylor and Barrett, 1985; Perry and Desforges, 2006). But, as shown by the following synopsis, the conclusions are equivocal.

## Previous hypotheses concerning the benefits to gas transfer at the gills

The first comprehensive study of the cardiorespiratory changes associated with aquatic hypoxia discovered that the transfer factor for oxygen across rainbow trout *Oncorhynchus mykiss* gills increased about fivefold during hypoxia (Randall et al., 1967). To explain the potential contribution of hypoxic bradycardia to this change, various mechanisms related to gill secondary lamellar blood perfusion have been proposed (see Taylor, 1985; Reid et al., 2006). Originally it was suggested that bradycardia decreased $\dot{Q}$ and the increased time that blood spent in gill lamellae conveyed the advantage of a longer diffusion time for gas exchange to occur (Satchell, 1960). However, because $\dot{Q}$ does not necessarily decrease in rainbow trout and the percentage of secondary lamellae perfused (equivalent to gill functional surface area) increases from 58% in normoxia to 71% during aquatic hypoxia (Booth, 1979), alternative mechanisms were needed. Randall (Randall, 1982) proposed that the increase in pulse pressure associated with bradycardia served to open poorly perfused vascular spaces in the gill lamellae, create a more even blood flow within the lamellae, and recruit unperfused lamellae (Farrell et al., 1979; Farrell et al., 1980), all of which increase the effective area for gas exchange. Vasoactive mechanisms also greatly influence lamellar perfusion patterns in fish (see Sundin, 1995). In particular, reflex cholinergic vasoconstriction of the proximal sections of the gill filamental arteries (Sundin and Nilsson, 1997) and an adrenergic systemic vasoconstriction (Wood and Shelton, 1980) are believed to enhance lamellar perfusion and oxygen uptake across the gills (Reid et al., 2006).

Two studies have examined the potential benefits of hypoxic bradycardia to oxygen uptake at dogfish gills, but they have produced conflicting results. In one study, cardiac vagotomy was used to prevent the 50% decrease in $f_H$ during hypoxia. However, gill diffusive conductance for oxygen was identical with and without bradycardia (Short et al., 1979). $\dot{Q}$ was unchanged by hypoxia and was the same in both intact and vagotomised fish. Similarly, vagotomy did not alter the 25–36% decrease in oxygen uptake during hypoxia, but it did decrease the oxygen partial pressure ($P_{O_2}$) of venous blood ($P\bar{v}_{O_2}$) during hypoxia from 1.5 kPa to 1.1 kPa. However, confounding the interpretation of these data was the finding that vagotomy also decreased (by 0.9–1.3 kPa) the routine normoxic oxygen tensions in arterial ($Pa_{O_2}$) and venous blood, as well as almost halving the respiratory response to hypoxia. As a result, respiratory stroke volume and the ventilation:perfusion ratio were also almost halved after vagotomy when compared with intact fish. In addition, hypoxia

did not increase either the transfer factor or the diffusing capacity for oxygen across the gills, even in intact dogfish.

Given the potentially confounding effects related to vagotomy and extensive instrumentation, a second study used atropine to pharmacologically block cardiac muscarinic receptors in less instrumented dogfish (Taylor and Barrett, 1985). In this study, atropine prevented the 50% decrease in $f_H$ during hypoxia, decreased gill diffusive conductance for oxygen by 28% and decreased $P_{a_{O_2}}$ by 0.8 kPa, indicating a decrease in the effectiveness of oxygen transfer into the blood. Contrary to the first study, this result was taken to indicate that hypoxic bradycardia improved gas exchange at the gills. As in the first study, $P_{v_{O_2}}$ similarly decreased to 1.1 kPa during hypoxia, but further comparisons between the two studies are complicated by the fact that oxygen uptake in the second study was over three times lower and did not decrease with hypoxia, and that $P_{a_{O_2}}$ levels were much lower during both normoxia (2.9 *vs* 12.0 kPa) and hypoxia (2.1 *vs* 4.3 kPa).

A pharmacological approach was also used to assess the effect of bradycardia and hypertension on gas transfer at the gills of rainbow trout (Perry and Desforges, 2006). Atropine injection largely attenuated a 35% decrease in $f_H$ associated with hypoxia, but the decrease in $P_{a_{O_2}}$ that accompanied hypoxia was identical with or without bradycardia. The authors suggested that hypoxic bradycardia *per se* provided no benefit to gill diffusive conductance for oxygen, especially since atropine had no effect on the hypoxia-mediated changes in ventilation. However, confounding factors following atropine administration were a 50% higher $\dot{Q}$ during both normoxia and hypoxia, and systemic hypertension, both of which could have affected gill perfusion. Consequently, rainbow trout were also treated with an α-adrenergic antagonist (prazosin), which almost halved systemic resistance and $P_{DA}$, and also restored the normoxic $\dot{Q}$, presumably because of reduced venous return to the heart. Unfortunately, prazosin also prevented the normal hyperventilatory response during hypoxia and caused $P_{DA}$ to collapse to just 0.67 kPa, so that eventually almost no oxygen was taken up into dorsal aortic blood. These confounding responses prevented the authors from providing a definitive conclusion regarding the role of hypoxic systemic hypertension on oxygen transfer at the gills.

The equivocal nature of these results for dogfish and rainbow trout leave the door somewhat open for further study of the potential benefits of bradycardia to gill gas exchange. Nevertheless, they highlight the experimental difficulties associated with seeking clearcut benefits of hypoxic bradycardia at the level of the gills. Given this unresolved state of affairs, the remainder of the manuscript creates a theoretical framework and provides evidence that hypoxic bradycardia could directly benefit the heart by improving myocardial function as it approaches a hypoxic state. What will become evident is that this topic is primed for further research.

## The importance of cardiac stroke volume in fishes

Regulation of $\dot{Q}$ in fishes differs in an important respect when compared with most air-breathing vertebrates. Many fishes have a remarkable ability to regulate $V_{SH}$ (increases of up to threefold are reported), although exceptions exist (Farrell, 1991; Farrell and Jones, 1992). Thus, while hypoxia produces profound decreases in $f_H$, large increases in cardiac stroke volume ($V_{SH}$) can maintain $\dot{Q}$ (Holeton and Randall, 1967; Short et al., 1979; Wood and Shelton, 1980; Fritsche and Nilsson, 1989). Some species do allow a modest decrease in $\dot{Q}$, depending on the level of hypoxia (see Perry and Desforges, 2006). In contrast, only a modest increase in $V_{SH}$ is possible in the mammalian heart because it normally operates close to the top arm of the Frank–Starling curve that describes the relationship between $V_{SH}$ and cardiac stretch (filling pressure). Instead, $f_H$ changes about threefold. The underlying cellular mechanism for the large operational range for $V_{SH}$ in rainbow trout is apparently an extended functional length–tension relationship related to a length-dependent increase in myofilament calcium sensitivity that allows active tension to increase at sarcomere lengths greater than those previously demonstrated for mammalian myocytes (Shiels et al., 2006). Even the primitive hagfish heart can increase $V_{SH}$ over threefold in response to filling pressure (Johnsson and Axelsson, 1996). Consequently, the Frank–Starling mechanism has deep evolutionary roots for the vertebrate heart, and hypoxic bradycardia in fishes exploits this large potential to increase $V_{SH}$ and maintain $\dot{Q}$.

## Improved contractility

This section develops the idea that a slower beating fish heart can generate more force to maintain $V_{SH}$ and therefore offset potential decreases in contractility due to the myocardium becoming hypoxic as $P_{v_{O_2}}$ decreases during hypoxia. When spontaneously beating isolated fish hearts are warmed, they beat at a higher rate and with a lower maximum force (Ask et al., 1981). A similar response is also seen when pacing frequency is increased for cardiac strips; maximum isometric tension decreases [termed a negative force–frequency effect (see Shiels et al., 2002)]. Consistent with this idea is the finding that maximal $V_{SH}$ is compromised under normoxic conditions in rainbow trout when $f_H$ is increased by either pacing (Farrell et al., 1988) or increasing temperature (Farrell et al., 1996). The opposite effect is seen in mammalian cardiac strips, where isometric tension increases with pacing frequency (Driedzic and Gesser, 1994). Thus, bradycardia allows fish hearts to potentially beat more forcefully and at the same time increases the diastolic filling time, which is a key determinant of end-diastolic cardiac volume. Conversely, if fish were to increase $f_H$ during hypoxia, the heart would potential suffer from decreased contractility and a reduced diastolic filling time, both of which would constrain $V_{SH}$.

These two benefits of hypoxic bradycardia would be particularly important when cardiomyocytes are approaching a hypoxic state, which has the well-described effect of depressing cardiac contractility (see Driedzic and Gesser, 1994; Hanson et al., 2006). However, such benefits assume that vagal

inhibition does not simultaneously result in a negative inotropic effect on ventricular muscle. This assumption seems valid given that cardiac vagal innervation is restricted to the pacemaker region and the atrium of fish (Nilsson, 1983). Even so, a mechanistic basis is still needed to explain exactly how a slower $f_H$ benefits myocardial contractility. A more complete elucidation of calcium handling in hypoxic myocytes might be useful in this regard.

### Reduction in whole animal and cardiac oxygen demands

In the 1970s, Taylor et al. suggested that hypoxic bradycardia in dogfish reduced cardiac work and decreased whole animal oxygen demand (Taylor et al., 1977). However, they incorrectly assumed that $\dot{Q}$ decreased. Furthermore, cardiac work is thought to be only a relatively small percentage (1–4%) of oxygen uptake for rainbow trout (Farrell and Steffensen, 1987), which means that very large savings in cardiac work are needed before they would be detectable in whole animal oxygen consumption.

Nevertheless, hypoxic bradycardia could reduce myocardial oxygen demand. Myocardial oxygen demand in fish is primarily determined by cardiac power output, which can be estimated from the product of $\dot{Q}$ and ventral aortic pressure ($P_{VA}$) (Farrell and Jones, 1992). Thus, with $\dot{Q}$ being maintained during hypoxia, any change in $P_{VA}$ provides an indication of potential alterations in myocardial oxygen demand. In lingcod *Ophiodon elongatus* and the hypoxia-tolerant traira *Hoplias malabraricus*, mean $P_{VA}$ progressively decreased with time and the degree of bradycardia during hypoxia (Farrell, 1982; Sundin et al., 1999). In Atlantic cod *Gadus morhua*, the initial increase in mean $P_{VA}$ subsided as hypoxic bradycardia developed. In fact, mean $P_{VA}$ remained elevated when hypoxic bradycardia was prevented by sectioning cranial nerves to the gills (Fritsche and Nilsson, 1989). These results suggest that hypoxic bradycardia lowered cardiac work and provided the benefit of a lowered myocardial oxygen demand. In contrast, in rainbow trout, mean $P_{VA}$ increased progressively with hypoxia (Holeton and Randall, 1967), suggesting that there may be no savings for the heart (but see below). This type of analysis should not be extended to other species using the more numerous measurements of $P_{DA}$ because $P_{DA}$ is not necessarily a good indicator of $P_{VA}$ since gill resistance can change independently of systemic resistance, although $P_{DA}$ is an important determinant of gill blood flow patterns.

An important secondary determinant of cardiac oxygen demand in mammals is the rate of pressure development (Suga et al., 1982; Suga, 1990). Thus, for a given level of stroke work, a heart consumes more oxygen when ejection pressure is elevated and stroke volume is small compared with when ejection pressure is lower and stroke volume is larger. This effect is much like walking *versus* running up the same mountain; the gain in potential energy remains the same in both situations, but running is metabolically more expensive. Consequently, hypoxic bradycardia could lower the rate of ventricular pressure development ($dP/dt$) simply by decreasing diastolic blood pressure, and thereby lower myocardial oxygen demand. Ventricular $dP/dt$ for hagfish hearts is about ten times slower than that for other fishes (Satchell, 1991) and normalized oxygen consumption (68 $\mu$mol $O_2$ $g^{-1}$ $h^{-1}$ $mW^{-1}$) (Forster, 1991) is 16% lower compared with rainbow trout (79 $\mu$mol $O_2$ $g^{-1}$ $h^{-1}$ $mW^{-1}$) (Farrell and Milligan, 1986), but whether or not the two are related is unknown. Myocardial efficiency also varies with power output, but how this might relate to hypoxic bradycardia is unknown.

### Enhanced myocardial oxygen delivery

The case made in this section is that hypoxic bradycardia, through several putative mechanisms, might improve myocardial oxygen delivery and prevent (or delay) myocardial hypoxia when $P\bar{v}_{O_2}$ decreases during hypoxia. If $Pa_{O_2}$ decreases and $\dot{Q}$ does not change, then tissue oxygen extraction from the blood must increase to maintain oxygen delivery to the tissues during hypoxia. Therefore, a profound decrease in $P\bar{v}_{O_2}$ is a characteristic feature of aquatic hypoxia in fishes. [Note: even when oxygen uptake is maintained, hypoxia may result in an oxygen deficit, given the observed increase in $\dot{Q}$ post-hypoxia for rainbow trout (Wood and Shelton, 1980).] To appreciate how a decrease in $P\bar{v}_{O_2}$ can greatly compromise myocardial oxygen supply (and how hypoxic bradycardia might offset this effect) requires a brief description of the considerable differences that exist between fish and mammals (as well as among fishes) with respect to the oxygen delivery systems that keep the cardiac muscle alive.

#### *Myocardial oxygen supply routes in water breathing fishes*

The most primitive arrangement of muscle fibres in the vertebrate heart [a Type I heart (Tota et al., 1983)] is entirely trabecular. Type I hearts are found in cyclostomes and most teleosts (Santer, 1985; Davie and Farrell, 1991), and the spongy myocardium of the atrium and ventricle is bathed by the venous blood contained within. Deep lacunae allow venous blood to almost reach the epicardial surfaces of the heart. This venous blood provides the oxygen supply of the heart (termed hereafter as the cardiac circulation).

The heart is the last major organ served by the circulatory system. Therefore, the amount of oxygen available in the venous blood that is available for the myocardium is predetermined to a large extent by systemic oxygen extraction. As such, oxygen supply to the spongy myocardium *via* the cardiac circulation is essentially the leftovers from other tissues. Some oxygen must always remain in venous blood, otherwise the heart cannot pump aerobically. Consequently, when $P\bar{v}_{O_2}$ decreases during hypoxia, this myocardial oxygen supply must be regarded as a precarious.

Some fish have a coronary circulation that supplies oxygenated arterial blood to the heart directly from the gills *via* the cephalad hypobranchial artery and/or the caudal subclavian and coracoid arteries. [Sharks and several ancient fishes have a cephalad supply, as do the small percentage of teleosts that possess a coronary supply (eels and marlin possess both

cephalad and caudal supplies). Rays, however, possess both caudal and cephalad coronaries, while *Latimeria* and some Chondrosteans have only a caudal supply.] Even so, the myocardial oxygen supply from the coronary circulation is generally only a supplement to the cardiac circulation. The coronary circulation primarily supplies the outer compact myocardium of the ventricle, which comprises as little as 10% in dogfish (Type III hearts) and 30–40% in trout (Type II hearts), but as much as 60% in tuna (Type IV hearts) (Tota et al., 1983; Santer, 1985; Tota, 1989; Farrell et al., 2007).

The coronary vessels of some fish (elasmobranchs, sturgeon and tuna) can reach the ventricular trabeculae [termed Thebesian vessels (Tota, 1989)]. Therefore, a theoretical case has been made that the spongy myocardium also benefits considerably from the coronary circulation, particularly in elasmobranchs (Tota, 1989). Lacking, however, is a quantification of the amount of blood flow supported by this Thebesian system. By comparison, the Thebesian vessels of the mammalian heart carry very little of the coronary blood flow. In addition, Tota has convincingly argued that an important metabolic zonation exists between the compact and the spongy myocardium in hypoxia-tolerant common carp *Cyprinus carpio* and athletic bluefin tuna *Thunnus thynnus thynnus* (Tota, 1983). His conclusion is largely based on several biochemical studies that have revealed differences between the two myocardial types, all of which point to very different blood and oxygen supplies [such as higher cristae density, mitochondrial enzyme activity, and lactate accumulation and oxidation rates, as well as a lower $K_m$ for succinate oxidase in the spongy myocardium, and a higher protein content in the compact myocardium; (Gemelli et al., 1980; Poupa et al., 1981; Greco et al., 1982; Kalous et al., 1989)]. A similar metabolic zonation, but perhaps less extreme, exists between the subepicardium and the subendocardium of the mammalian heart (Tota, 1983), and the subendocardium is considered by some to be in a permanent condition of a lower $P_{O_2}$ (see Tota, 1983). This metabolic zonation seems to indirectly argue against a substantial coronary oxygen supply to the spongy myocardium of fishes. Nevertheless, the possibility does exist that the metabolic zonation is unrelated to the blood supply. Furthermore, it is unclear whether metabolic zonation applies to the elasmobranch heart.

In teleosts such as rainbow trout (Farrell et al., 1988) and the air-breathing Pacific tarpon *Megalops cyprinoides* (Farrell et al., 2007), the coronary circulation is restricted to the outer compact myocardium by a connective tissue layer between it and the spongy myocardium. Therefore, the only way the spongy myocardium of the ventricle could benefit from the coronary circulation is through oxygen left in the coronary veins, which drain into the heart near the atrio-ventricular region.

### *Depleting the oxygen supply to the spongy myocardium*

The decrease in $P\mathrm{v}_{O_2}$ with hypoxia (and exercise) means that at some point oxygen delivery to cardiac trabeculae will become compromised. Calculations suggest that myocardial oxygen consumption hardly changes the oxygen content of venous blood in rainbow trout (Farrell, 1987) and so it is $P\mathrm{v}_{O_2}$ that sets the myocardial oxygen delivery rate. This conclusion is supported by the fact that anemic flounder maintain $\dot{Q}$ even when $P\mathrm{v}_{O_2}$ is reduced to 0.8 kPa (Wood et al., 1979a).

The threshold $P\mathrm{v}_{O_2}$ below which the spongy myocardium becomes hypoxic is unknown. However, minimum values for $P\mathrm{v}_{O_2}$ during hypoxia and exercise range from 0.8 to 2.1 kPa (Davie and Farrell, 1991). The threshold $P\mathrm{v}_{O_2}$ will obviously depend on myocardial oxygen demand (equivalent to power output), and so when myocardial oxygen demand is elevated during exercise and at warm temperature, the $P\mathrm{v}_{O_2}$ threshold is expected to be at the higher end of the range (e.g. Farrell and Clutterham, 2003). During hypoxia, however, when $\dot{Q}$ is either maintained or reduced, the $P\mathrm{v}_{O_2}$ threshold is expected to be near the lower end of the range. Indeed, hypoxic dogfish reduced $P\mathrm{v}_{O_2}$ to 1.1–1.5 kPa (Short et al., 1979; Taylor and Barrett, 1985). Similarly, $P\mathrm{v}_{O_2}$ decreased from 2.7 to 0.8 kPa as rainbow trout progressively decreased $f_H$ from 75 $\mathrm{min}^{-1}$ to 22 $\mathrm{min}^{-1}$ (Holeton and Randall, 1967). This background information on myocardial oxygen supply places the potential benefits of hypoxic bradycardia in a clearer light.

### *Potential benefits of hypoxic bradycardia to the trabecular oxygen supply*

By lengthening the diastolic period and increasing $V_{SH}$, hypoxic bradycardia offers two potential advantages in terms of oxygen delivery to a Type I heart.

(a) Hypoxic bradycardia increases blood residence time in the lumen of the heart, which then favours oxygen extraction (Farrell, 1984). Blood residence time in the lumen of the heart will become a critical factor whenever the threshold $P\mathrm{v}_{O_2}$ is reached during hypoxia. This benefit is particularly important for the trabecular fish heart because ventricular end-systolic volume (ESV) is much lower than that in mammals, so that the trabeculae become compressed against each other during systole, temporarily increasing the effective oxygen diffusion distance beyond that of a single trabecula. If ESV is zero, oxygen extraction could be prevented altogether. Qualitative studies using angiographic cardiac imaging suggest that ESV is routinely close to zero [e.g. for anaesthetised *Channa argus* (Andresen et al., 1987)]. For the anaesthetised leopard shark *Triakis semifasciata*, mean ejection fraction was measured as 80%, ranging between 62% and 92%, using echo-cardiography (Lai et al., 1990). A thorough and controlled study with echotomographic images of working, perfused *in situ* rainbow trout hearts found that ESV was zero for a routine $V_{SH}$, but increased at maximum $V_{SH}$ (Franklin and Davie, 1992); end-diastolic volume (EDV) increased over the whole range of cardiac filling pressures. Thus, the majority of oxygen exchange between cardiac circulation and the trabeculae must routinely occur during diastole. Furthermore, the large increases in $V_{SH}$ associated with hypoxic bradycardia should increase ESV and thus extend the time available for oxygen diffusion into the spongy myocardium. With an increase in cardiac output pressure ($=P_{VA}$), both EDV and ESV increased

in the *in situ* heart (Franklin and Davie, 1992). This is an interesting discovery because $P_{VA}$ increased during hypoxia in rainbow trout, which could then increase ESV and promote oxygen diffusion from the cardiac circulation during systole. Further studies involving *in vivo* imaging of fish hearts would provide greater insight into such possibilities.

(b) A substantial increase in end-diastolic volume with the increased $V_{SH}$ would stretch and decrease the diameter of the trabeculae, as well as promote the mixing of blood within the lumen itself. Such changes would reduce the effective oxygen diffusion distance.

The putative benefits alluded to above would also be favourable for anemic fish under normoxic conditions. Anemia can induce a two- to threefold increase in $V_{SH}$ without any change in $f_H$ and oxygen uptake, while $P\bar{v}_{O_2}$ decreases to a level close to the suggested threshold for adequate myocardial oxygen delivery [0.9 kPa in starry flounder *Platichthys stellatus* (Wood et al., 1979a) and 0.9 kPa in rainbow trout (Holeton, 1971a), but 3.3 kPa in rainbow trout (Cameron and Davis, 1970)]. Even so, the cardiac control mechanisms during anemia will likely differ from those during hypoxia because anemia produces a large decrease in systemic vascular resistance, unlike aquatic hypoxia. In addition, modest anemia reverses both the hypertension and bradycardia in hypoxic rainbow trout (Wood and Shelton, 1980).

The ventricle has a considerably greater muscle mass than in the atrium and its trabeculae are likely thicker. While a thicker trabecula can develop more tension (to generate higher blood pressures), the diffusion distance to its centre is greater. Thus, the maximum diameter of an individual ventricular trabecula represents a fine design balance between the considerations for tension development and oxygen diffusion (Davie and Farrell, 1991). As such, cardiac growth represents a further challenge to myocardial oxygen supply. Consistent with this challenge is the observed increase in the proportion of compact myocardium (i.e. supplied with a coronary circulation) in larger Atlantic salmon *Salmo salar* [Type II heart (Poupa et al., 1974)] and Pacific tarpon [an air-breathing fish with a Type II heart (Farrell et al., 2007)]. Furthermore, the myoglobin concentration in the bluefin tuna heart almost quadruples with growth from 0.5 to 50 kg, and its accumulation rate accelerates when tuna reach ~20 kg (Poupa et al., 1981). All of these developmental changes point to compensations that secure a better myocardial oxygen supply.

The converse of this reasoning is that myocardial oxygen delivery should be favoured in larval fish for at least two reasons. One reason is that cardiac dimensions are smaller and diffusion distances are shorter. The other reason is that an appreciable proportion of the larval fish oxygen uptake occurs across the skin (Rombough, 1988), which could have an advantage of increasing $P\bar{v}_{O_2}$. Thus, the finding that neither rainbow trout (Holeton, 1971b) nor Arctic char *Salvelinus alpinus* (McDonald and McMahon, 1977) hatchlings respond to aquatic hypoxia with bradycardia is perhaps not surprising. Rainbow trout actually responded to hypoxia with tachycardia until day 9 post-hatch, after which they responded with bradycardia. In char, a similar tachycardia response persisted until day 47 post-hatch, at which time the gill lamellar surface was estimated to be one quarter of that needed to support the entire oxygen uptake of the fish. These results also can be explained by a delay in the ontogenetic development of the reflex arc for hypoxic bradycardia, but then it is curious why the cardio-accelerator reflex develops before the cardio-inhibitory one. Examining the contribution of skin oxygen uptake to the myocardial oxygen supply in larval fish would be a challenging but not impossible experiment to perform. For example, experiments with zebrafish *Danio rerio* have already shown that rearing hatchlings in chronic hypoxia elevates $f_H$ at day 5 post-fertilisation, shortly after which functional anemia (either CO or phenylhydrazine treatment) decreased $f_H$ (Jacob et al., 2002).

### *The role of the coronary circulation*

Compared with the cardiac circulation, the coronary circulation offers a more secure myocardial oxygen supply at a higher $P_{O_2}$. The coronary circulation appeared early in the evolution of the vertebrate heart [i.e. it is present in elasmobranchs (Santer, 1985; Tota, 1989)]. Likely, natural selection favoured a coronary circulation in situations where the cardiac circulation is less reliable (aquatic hypoxia and exercise). The evolutionary progression toward frequency modulation for the vertebrate heart and greater blood pressure generation (Farrell, 1991) appeared later than the elasmobranchs.

The importance of coronary blood flow to myocardial oxygen supply has been clearly demonstrated by the increase in coronary blood flow observed during hypoxia (and exercise) in rainbow trout and coho salmon *Oncorhynchus kitsutch* (Axelsson and Farrell, 1993; Gamperl et al., 1995). Why then is there still a need for hypoxic bradycardia (as in dogfish, rainbow trout and tunas) to improve myocardial oxygen supply? One possibility is that the overall benefit of the coronary circulation to the entire heart is relatively small, which may be the case for species such as the dogfish that can have as little as 10% compact myocardium (Farrell et al., 2007). Salmonids can actually live and swim with the coronary circulation completed ligated, but this at the expense of cardiac pressure generation (see Steffensen and Farrell, 1998).

It is also likely that hypoxic bradycardia benefits coronary blood flow *per se*. In mammals, coronary blood flow occurs only during diastole because ventricular contraction compresses coronary vessels. A similar situation exists in coho salmon with coronary blood flow being greatly reduced during systole (Axelsson and Farrell, 1993). Therefore peak coronary blood flow, and hence myocardial oxygen delivery, occur during diastole. Consequently, any increase in the diastolic period associated with hypoxic bradycardia has a direct benefit to the myocardial oxygen supply. Again fish would benefit more from this phenomenon than mammals because of an important difference between fish and mammals in terms of the coronary arterial perfusion pressure. The mammalian heart has coronaries that are derived at the root of the aorta and so

coronary arterial perfusion pressure almost exactly matches the pressures developed by the ventricle itself. This is not true for fish because of the post-branchial origin of the coronaries. In fact, coronary perfusion pressure will be 25–35% lower than $P_{VA}$ and so ventricular systole possibly has a greater impact on coronary blood flow in fishes than in mammals.

### *Hypoxia tolerance in fishes*

The potential for fish hearts to operate anaerobically to meet any oxygen shortfall would negate much of the proceeding discussion. A recent analysis concluded, based on rather limited data available, that maximum cardiac glycolytic ATP turnover rates do not differ greatly between anoxia-tolerant and hypoxia-sensitive species and therefore could not fully explain anoxia tolerance among vertebrates (Farrell and Stecyk, 2007). Instead, myocardial anoxia tolerance was suggested to be based on two strategies: either routine cardiac ATP demand is lower than the maximum cardiac glycolytic ATP turnover rate (e.g. in hagfishes and crucian carp *Carassius carassius*), or routine cardiac ATP demand is downregulated to well below the maximum cardiac glycolytic ATP turnover rate (e.g. in common carp *Cyprinus carpio* and freshwater turtles). Consequently, definitive statements on the myocardial oxygen requirement during hypoxia cannot be made with confidence without proper knowledge of routine cardiac power output (= cardiac ATP demand) and maximum cardiac glycolytic ATP turnover rate. On the one hand, it is not necessary to activate bradycardia if glycolytic ATP production is sufficient and the heart can operate normally during anoxia, which is the case for crucian carp (Stecyk et al., 2004), provided waste products are dealt with. On the other hand, hypoxic bradycardia can greatly depress cardiac power output and ATP requirement to bring it within a level that can be supported by glycolyic ATP production, which then eliminates the need for oxygen for several hours, as in the case of common carp. This situation may also apply to the hypoxia-tolerant epaulette shark *Hemiscyllium ocellatum* (Stensløkken et al., 2004). A depressed cardiac state is suggested by marked decreases in $f_H$, $P_{VA}$ and $P_{DA}$, and reduced blood velocity in gill blood vessels. Much work lies ahead in characterizing the role that cardiac glycolysis plays in supporting cardiac activity, and measurements of $\dot{Q}$ and $P_{VA}$ to estimate cardiac power output during hypoxia will greatly benefit such evaluation.

### *The benefit of air breathing to myocardial oxygen supply in fish*

The majority of air-breathing fishes mix oxygenated blood from the air breathing organ (ABO) into systemic venous return (Satchell, 1976; Olson, 1994; Graham, 1997). Consequently, an important consequence of air breathing is an increase in $P_{VO_2}$ of the cardiac circulation. Farmer (Farmer, 1997) went further to suggest that the evolution of air breathing among fishes was to provide a more secure myocardial oxygen supply during exercise. All the same, air-breathing frequency can increase appreciably during aquatic hypoxia without increasing during exercise alone (Seymour et al., 2007).

Given that air breathing provides a more secure myocardial oxygen supply, the preceding discussion sets up the expectation that hypoxic bradycardia is redundant in air-breathing fishes faced with aquatic hypoxia. This appears to be the case for lungfishes, since representatives of all three genera maintained $f_H$ during aquatic hypoxia [*Neoceratodus* (Fritsche et al., 1993); *Lepidosiren* (Sanchez et al., 2001); *Protopterus* (Perry et al., 2005)]. Similarly, garfish *Lepisosteus oculatus* (Smatresk and Cameron, 1982) showed modest tachycardia rather than bradycardia during an exposure to aquatic hypoxia that was severe enough to initiate air breathing. In contrast, the jeju *Hoploerythrinus unitaeniatus* developed hypoxic bradycardia, increased gill ventilation volume, and increased $P_{DA}$ as water became sufficiently hypoxic to compromise oxygen uptake, despite an increase in air-breathing frequency (Oliveira et al., 2004). In the absence of other $f_H$ measurements during prolonged aquatic hypoxia, it is impossible to determine to what extent other air-breathing fishes may have lost the hypoxic bradycardia response. Even so, the absence of hypoxic bradycardia in lungfishes could be completely unrelated to myocardial oxygen delivery. For example, separation of the oxygenated and deoxygenated streams of blood is crucial within the single chamber of the lungfish ventricle but may be particularly susceptible to alterations in $f_H$ and is best achieved within a certain $f_H$ range. Likewise, routine vagal tone is very low in *Lepidosiren* and this may preclude tachycardia (Axelsson et al., 1989).

The tachycardia associated with an air breath and the subsequent decrease in $f_H$ during breath holding (Johansen, 1970) could be viewed as a response to progressive hypoxia (i.e. oxygen being removed from the ABO). However, Graham suggested that the prevalence of tachycardia associated with air breathing has probably been overstated (Graham, 1997). Notable exceptions include all the lungfish genera, *Lepisosteus oculatus*, *Amia calva* and *Arapaima gigas* [see table 6.5 in Graham (Graham, 1997)].

Thus, further work is needed to test the possibility that the loss of hypoxic bradycardia coincided with a benefit to myocardial oxygen supply through air breathing. In addition, chronotropic responses in air-breathing fishes will need to be placed in proper context with the changes in ABO blood flow, the autonomic control of the heart and the state of the ABO (Graham, 1997). For example, inflations and deflations of the ABO by themselves can increase and decrease $f_H$ in air-breathing fishes (Graham, 1997). Also, blood flow to the ABO must increase to ensure appropriate ventilation:perfusion matching (Johansen, 1970; Burggren et al., 1997), but ABO blood flow can increase through either a redistribution of blood flow from other tissues, which requires no change in $\dot{Q}$, or an increase in $\dot{Q}$ which could easily override the drive for hypoxic bradycardia. *Amia calvens*, for example, showed no change in $f_H$ with air breathing (Johansen et al., 1970) and increased $\dot{Q}$ solely by increased ABO blood flow (Randall et al., 1981). In contrast, jeju increased both $f_H$ and ABO blood flow after an air breath, but without changing $\dot{Q}$ (Farrell, 1978). Even the responses to emergence, which induces either no change or a

decrease in $f_H$ among air-breathing fishes [table 6.4 in Graham (Graham, 1997)], need careful consideration. For example, both the jeju and the piraracu *Arapaima gigas* decreased $f_H$ during a brief air exposure, but this response need not have been hypoxic bradycardia, especially if gill secondary lamellae had collapsed in air and greatly increased branchial vascular resistance (Farrell, 1978).

### *Temperature*

Changes in acclimation temperature have important effects on the hemoglobin oxygen dissociation curve, as well as whole animal and cardiac oxygen demands. While confounding broad comparisons between tropical air-breathing and temperate water-breathing fishes, such temperature effects can still provide valuable clues to the potential benefits of hypoxic bradycardia.

Though rarely mentioned, the right-shift in the oxygen dissociation curve with warm temperature has the distinct benefit to the heart of increasing $P_{\mathrm{V}O_2}$ for a given tissue oxygen extraction (or possibly maintaining $P_{\mathrm{V}O_2}$ if tissue oxygen extraction increases). [In fact, natural selection for haemoglobins with strong temperature coefficients and Bohr effects could be related to such a benefit.] Given that both the overall myocardial oxygen demand and the threshold $P_{\mathrm{V}O_2}$ increase with temperature (Hanson and Farrell, 2007), the right-shift in the oxygen dissociation curve is all the more beneficial.

Consistent with this thinking is the observation that hypoxic bradycardia was more marked in dogfish at 17°C compared with 12°C and 7°C (Butler and Taylor, 1975), and at the same time myocardial power output was three times higher. However, this interpretation is confounded by the consideration that myocardial power output at 7°C approached a level known to be close to the maximum glycolytic capacity of fish hearts (Farrell and Stecyk, 2007). In fact, even the hypoxia-sensitive rainbow trout can withstand brief periods of complete anoxia at 5°C because of a low cardiac power output (see Overgaard et al., 2004). Regardless of the exact explanation, a reduced myocardial oxygen demand was associated with the loss of hypoxic bradycardia in cold-acclimated dogfish.

Temperature acclimation also alters routine cardiac vagal tone, increasing it with temperature in dogfish (Taylor et al., 1977) and decreasing it with temperature in rainbow trout (Wood et al., 1979b). Slowing the pacemaker rate at high temperature has a potential to benefit the dogfish myocardium, but the observations for rainbow trout are more difficult to reconcile. Information on how hypoxic bradycardia and coronary blood flow vary with temperature in rainbow trout certainly would be useful for the evaluation. However, we do know that temperature acclimation resets the cardiac pacemaker rate in teleost fish, including rainbow trout (Farrell, 1991), compensating for the direct effect of temperature on $f_H$. In addition, atrial myocytes from cold-acclimated rainbow trout have a higher resting membrane potential (Shiels et al., 2000), which can be lowered and stabilized by an acetylcholine-activated potassium current (Molina et al., 2007). Consequently, the higher vagal tone in cold-acclimated rainbow trout may be key to stabilizing $f_H$, especially since cardiac arrhythmias have been observed in cold-acclimated perfused rainbow trout hearts (Graham and Farrell, 1989).

Cold temperature suppression of whole animal and myocardial metabolic rates might explanation why Antarctic fishes do not display hypoxic bradycardia (see Axelsson, 2006). Routine $V_{SH}$ is unusually high in Antarctic fishes compared with temperate species. This reflects the need for a high $\dot{Q}$ to compensate for reduced and even negligible haemoglobin in the blood (see Sidell and O'Brien, 2006) and a low $f_H$ resulting from an extremely high resting cardiac vagal tone in some Antarctic fishes (Axelsson et al., 1992). The net result is a greatly elevated cardiac stroke work (see Axelsson, 2006), which is exactly the situation produced by hypoxic bradycardia (but to a lesser degree). Given this parallel, perhaps some Antarctic fish hearts should be considered to be in an 'adapted hypoxic state', especially since they can have negligible myoglobin in their hearts (Sidell and O'Brien, 2006) and lack a coronary circulation (Tota et al., 1988; Axelsson, 2006). The finding that myocytes of the haemoglobinless icefish have low myofibrilar and high mitochondrial contents (Tota et al., 1988) points to a low oxygen demand and a poor oxygen supply. Furthermore, the reduced oxygen carrying capacity of their blood raises the possibility that oxygen extraction by the myocardium can significantly decrease the oxygen content of blood passing through the heart, unlike temperate species with their high haemoglobin concentration. In fact, the myocardial oxygen supply may be so precariously balanced in Antarctic fishes, that it may be essential that exercise capacity is limited so that $P_{\mathrm{V}O_2}$ is not depressed below critical levels through substantial increases in tissue oxygen extraction.

## Conclusions

The sensing and processing of hypoxic signals, the responses to these hypoxic signals and their modulation by other physical and physiological factors are an immense topic with numerous novel and exciting discoveries. The question posed here represents a small topic in this arena and, as such, cannot include an all-encompassing review on the topic of hypoxia in fishes. Some topics were not included because they were considered secondary to the main question. For example, hypoxia sensing by the gills is clearly linked to the release of catecholamines from chromaffin tissue into the plasma (see Reid and Perry, 2003), which among other benefits would provide important protection of fish cardiac tissue from the negative inotropic effects of hypoxia as well as exert positive chronotropic effects (see Hanson et al., 2006). However, in rainbow trout at least, the cardiorespiratory adjustments appear to occur well before catecholamines are released into the circulation (Reid and Perry, 2003), and therefore the benefits would be secondary to the initiation of hypoxic bradycardia.

The main thesis developed here is that hypoxic bradycardia can afford a number of direct benefits to the fish heart largely because the oxygen supply to the spongy myocardium is

precarious. In the first place, the unusual mechanical properties of the fish heart allow for large increases in $V_{SH}$ to compensate for bradycardia and maintain $\dot{Q}$. Among the potential benefits of hypoxic bradycardia is an increase in the diastolic residence time of blood in the lumen of the heart, which offers the advantage of increased time for diffusion. The increase in $V_{SH}$ will stretch the cardiac chambers, potentially reducing the diffusion distance for oxygen. Slowing $f_H$ could improve cardiac contractility through the negative force–frequency effect. Hypoxic bradycardia could also reduce cardiac oxygen demand by reducing cardiac d$P$/d$t$ and cardiac power output, something that could be masked at cold temperatures because of the reduced myocardial workload. The presence of a coronary circulation in certain fishes decreases the reliance of the heart on $P_{VO_2}$, and hypoxic bradycardia may benefit oxygen delivery *via* an extended diastolic period, during which there is peak coronary blood flow. In contrast, there is a notable absence of hypoxic bradycardia in fishes that breathe air during aquatic hypoxia and raise their $P_{VO_2}$. Still needed to test these ideas are carefully conducted experiments that properly characterise potential benefits in terms of myocardial oxygen supply and demand, and also consider the ability of the fish heart to support its routine cardiac power output through glycolysis. In the absence of such experiments, the putative mechanisms listed above could represent associations rather than cause-effect relationships. But given the lack of an autonomic wiring system for hypoxic bradycardia in hagfishes and that their cardiac glycolytic ATP turnover rate is adequate for routine cardiac function (Forster et al., 1991; Farrell, 1991), a great starting place might be to characterise the lamprey's cardiovascular response to aquatic hypoxia, given their unique excitatory vagal cardiac innervation.

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